# WEST Search History

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DATE: Tuesday, July 12, 2005

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| | *DB=PGPB; THES=ASSIGNEE; PLUR=YES; OP=ADJ* | | |
| ☑ | L8 | L4 | 28 |
| | *DB=USPT; THES=ASSIGNEE; PLUR=YES; OP=ADJ* | | |
| ☑ | L7 | L4 | 30 |
| | *DB=PGPB,USPT,EPAB,JPAB,DWPI; THES=ASSIGNEE; PLUR=YES; OP=ADJ* | | |
| ☑ | L6 | L4 and (ankara modified virus) | 1 |
| ☑ | L5 | L4 and avipox | 1 |
| ☑ | L4 | L3 and vaccinia | 58 |
| ☑ | L3 | L2 and vector | 94 |
| ☐ | L2 | L1 and envelope protein E1 | 129 |
| ☐ | L1 | HCv | 6460 |

END OF SEARCH HISTORY

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                  data from INPADOC
 NEWS  4  FEB 28  BABS - Current-awareness alerts (SDIs) available
 NEWS  5  MAR 02  GBFULL: New full-text patent database on STN
 NEWS  6  MAR 03  REGISTRY/ZREGISTRY - Sequence annotations enhanced
 NEWS  7  MAR 03  MEDLINE file segment of TOXCENTER reloaded
 NEWS  8  MAR 22  KOREAPAT now updated monthly; patent information enhanced
 NEWS  9  MAR 22  Original IDE display format returns to REGISTRY/ZREGISTRY
 NEWS  10 MAR 22  PATDPASPC - New patent database available
 NEWS  11 MAR 22  REGISTRY/ZREGISTRY enhanced with experimental property tags
 NEWS  12 APR 04  EPFULL enhanced with additional patent information and new
                  fields
 NEWS  13 APR 04  EMBASE - Database reloaded and enhanced
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                  based on application date in CA/CAplus and USPATFULL/USPAT2
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                  applications.
 NEWS  16 APR 28  Improved searching of U.S. Patent Classifications for
                  U.S. patent records in CA/CAplus
 NEWS  17 MAY 23  GBFULL enhanced with patent drawing images
 NEWS  18 MAY 23  REGISTRY has been enhanced with source information from
                  CHEMCATS
 NEWS  19 JUN 06  The Analysis Edition of STN Express with Discover!
                  (Version 8.0 for Windows) now available
 NEWS  20 JUN 13  RUSSIAPAT: New full-text patent database on STN
 NEWS  21 JUN 13  FRFULL enhanced with patent drawing images
 NEWS  22 JUN 27  MARPAT displays enhanced with expanded G-group definitions
                  and text labels
 NEWS  23 JUL 01  MEDICONF removed from STN
 NEWS  24 JUL 07  STN Patent Forums to be held in July 2005

 NEWS EXPRESS  JUNE 13 CURRENT WINDOWS VERSION IS V8.0, CURRENT
               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 13 JUNE 2005

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=> "hepatitits C envelope protein E1"
             1 "HEPATITITS"
       3326456 "C"
         51183 "ENVELOPE"
          9134 "ENVELOPES"
         56544 "ENVELOPE"
                 ("ENVELOPE" OR "ENVELOPES")
       1761714 "PROTEIN"
       1225322 "PROTEINS"
       2047023 "PROTEIN"
                 ("PROTEIN" OR "PROTEINS")
         35928 "E1"
L1           0 "HEPATITITS C ENVELOPE PROTEIN E1"
                 ("HEPATITITS"(W)"C"(W)"ENVELOPE"(W)"PROTEIN"(W)"E1")

=> "hepatitis C virus envelope"
         48119 "HEPATITIS"
             1 "HEPATITISES"
         48120 "HEPATITIS"
                 ("HEPATITIS" OR "HEPATITISES")
       3326456 "C"
        321999 "VIRUS"
         68677 "VIRUSES"
        333808 "VIRUS"
                 ("VIRUS" OR "VIRUSES")
         51183 "ENVELOPE"
          9134 "ENVELOPES"
         56544 "ENVELOPE"
                 ("ENVELOPE" OR "ENVELOPES")
L2         163 "HEPATITIS C VIRUS ENVELOPE"
                 ("HEPATITIS"(W)"C"(W)"VIRUS"(W)"ENVELOPE")

=> HCV
          9348 HCV
            18 HCVS
L3        9352 HCV
```

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                (HCV OR HCVS)

=> envelope
         51183 ENVELOPE
          9134 ENVELOPES
L4       56544 ENVELOPE
                (ENVELOPE OR ENVELOPES)

=> L3 and L4
L5         674 L3 AND L4

=> recombinant and L5
        173012 RECOMBINANT
          6652 RECOMBINANTS
        176631 RECOMBINANT
                (RECOMBINANT OR RECOMBINANTS)
L6         154 RECOMBINANT AND L5

=> vector and L6
        145854 VECTOR
         89478 VECTORS
        198198 VECTOR
                (VECTOR OR VECTORS)
L7          53 VECTOR AND L6

=> rVV and L7
           288 RVV
            42 RVVS
           300 RVV
                (RVV OR RVVS)
L8           0 RVV AND L7

=> vaccinia and L7
          9963 VACCINIA
             2 VACCINIAS
          9964 VACCINIA
                (VACCINIA OR VACCINIAS)
L9           9 VACCINIA AND L7

=> truncated and L8
         32137 TRUNCATED
L10          0 TRUNCATED AND L8

=> truncated and L7'
         32137 TRUNCATED
L11          5 TRUNCATED AND L7

=> glycosylation and L7
         31763 GLYCOSYLATION
           503 GLYCOSYLATIONS
         31896 GLYCOSYLATION
                (GLYCOSYLATION OR GLYCOSYLATIONS)
L12          5 GLYCOSYLATION AND L7

=> mutated and L6
         28228 MUTATED
L13          1 MUTATED AND L6

=> truncated and l6
         32137 TRUNCATED
L14         15 TRUNCATED AND L6

=> D L14 IBIB ABS 1-14

L14  ANSWER 1 OF 15  CAPLUS  COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER:        2005:163200  CAPLUS
TITLE:                   Purification and application of C-terminally
                         truncated hepatitis C virus E1 proteins
                         expressed in Escherichia coli
```

AUTHOR(S): Liu, Jing; Zhu, Li-Xin; Kong, Yu-Ying; Li, Guang-Di; Wang, Yuan
CORPORATE SOURCE: State Key Laboratory of Molecular Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, 200031, Peop. Rep. China
SOURCE: World Journal of Gastroenterology (2005), 11(4), 503-507
CODEN: WJGAF2; ISSN: 1007-9327
PUBLISHER: World Journal of Gastroenterology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB AIM: To explore the possibility of expressing hepatitis C virus ( **HCV**) **envelope** protein 1 (E1) in Escherichia coli (E coli) and to test the purified **recombinant** E1 proteins for clin. and research applications. METHODS: C-terminally **truncated** E1 fragments were expressed in E. coli as hexa-histidine-tagged fusion proteins. The expression products were purified under denaturing conditions using immobilized-metal affinity chromatog. Purified E1 proteins were used to immunize rabbits. Rabbit anti-sera thus obtained were reacted with both E. coli- and mammalian cell-expressed E1 glycoproteins as detected by Western blot. RESULTS: Full-length E1 protein proved difficult to express in E. coli. C-terminally **truncated** E1 was successfully expressed in E. coli as hexa-histidine-tagged **recombinant** fusion protein and was purified under denaturing conditions on Ni2+-NTA agarose. Rabbit anti-sera raised against purified **recombinant** E1 specifically reacted with mammalian cell-expressed E1 glycoproteins in Western blot. Furthermore, E. coli-derived E1 protein was able to detect animal antibodies elicited by E1-based DNA immunization. CONCLUSION: These results demonstrate that the prokaryotically expressed E1 proteins share identical epitopes with eukaryotically expressed E1 glycoprotein. The E. coli-derived E1 proteins and corresponding antisera can become useful tools in anti-**HCV** vaccine research.

REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 2 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2003:758562 CAPLUS
DOCUMENT NUMBER: 140:25396
TITLE: CD81-dependent binding of hepatitis C virus E1E2 heterodimers
AUTHOR(S): Cocquerel, Laurence; Kuo, Chiung-Chi; Dubuisson, Jean; Levy, Shoshana
CORPORATE SOURCE: Department of Medicine/Division of Oncology, Stanford University Medical Center, Stanford, CA, 94305, USA
SOURCE: Journal of Virology (2003), 77(19), 10677-10683
CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Hepatitis C virus (**HCV**) is the leading cause of chronic liver disease worldwide. **HCV** is also the major cause of mixed cryoglobulinemia, a B-lymphocyte proliferative disorder. Direct experimentation with native viral proteins is not feasible. **Truncated** versions of **recombinant** E2 **envelope** proteins, used as surrogates for viral particles, were shown to bind specifically to human CD81. However, **truncated** E2 may not fully mimic the surface of **HCV** virions because the virus encodes 2 **envelope** glycoproteins that associate with each other as E1E2 heterodimers. Here we show that E1E2 complexes efficiently bind to CD81 whereas **truncated** E2 is a weak binder, suggesting that **truncated** E2 is probably not the best tool with which to study cellular interactions. To gain better insight into virus-cell interactions, we developed a method by which to isolate E1E2 complexes that are properly folded. We demonstrate that purified E1E2 heterodimers bind to cells in a CD81-dependent manner. Furthermore, engagement of B cells by purified E1E2 heterodimers results in their aggregation and in protein tyrosine phosphorylation, a hallmark of B-cell activation. These

studies provide a possible clue to the etiol. of **HCV**-associated B-cell lymphoproliferative diseases. They also delineate a method by which to isolate biol. functional E1E2 complexes for the study of virus-host cell interaction in other cell types.

REFERENCE COUNT: 60 THERE ARE 60 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 3 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2002:180436 CAPLUS
DOCUMENT NUMBER: 137:227162
TITLE: Cloning and expression of human CD81 major extracellular loop in E. coli and its activity
AUTHOR(S): Zhang, Guojun; Ling, Shigan; Song, Xiaoguo; Zhang, Heqiu; Chen, Kun; Zhu, Cuixia; Xiu, Bingshui
CORPORATE SOURCE: Institute of Basic Medical Sciences, Academy of Military Medical Sciences, Beijing, 100850, Peop. Rep. China
SOURCE: Junshi Yixue Kexueyuan Yuankan (2001), 25(4), 260-264
CODEN: JYKYEL; ISSN: 1000-5501
PUBLISHER: Junshi Yixue Kexueyuan Yuankan Bianjibu
DOCUMENT TYPE: Journal
LANGUAGE: Chinese

AB An expression plasmid for a fusion protein of human CD81 major extracellular loop was constructed and binding activity of its expressed protein with **HCV** E2 was studied. CD81 major extracellular loop sequence was amplified from human peripheral blood lymphocytes by RT-PCR, then inserted into the expression vector pBVIL1, and expressed in E. coli. The purified fusion protein was tested for binding activity with E2. CD81-EC2 gene was correctly amplified and inserted into the vector as confirmed by sequencing. The preliminary study showed that the **recombinant** CD81/EC2 could bind **truncated HCV** E2 (384-661) protein expressed in E. coli. This work proved the way for further study on interactions of CD81 with **HCV** and its E2, and for preparation of anti-EC2 monoclonal antibody.

L14 ANSWER 4 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2001:912910 CAPLUS
DOCUMENT NUMBER: 137:104371
TITLE: Secretory expression of different C-terminal **truncated HCV** E1 proteins in mammalian cells and characterization of the expressed products
AUTHOR(S): Zhu, Jun; Kong, Yuying; Liu, Jing; Zhang, Zuchuan; Wang, Yuan; Li, Guangdi
CORPORATE SOURCE: Institute of Biochemistry and Cell Biology, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, Shanghai, 200031, Peop. Rep. China
SOURCE: Shengwu Huaxue Yu Shengwu Wuli Xuebao (2001), 33(6), 634-640
CODEN: SHWPAU; ISSN: 0582-9879
PUBLISHER: Shanghai Kexue Jishu Chubanshe
DOCUMENT TYPE: Journal
LANGUAGE: Chinese

AB Three fragments of **HCV envelope** 1 (E1) with different C-terminal truncation at aa310, aa325, aa340 were cloned into the mammalian expression vector pSecTagB. An epitope in the hepatitis B surface antigen, preS1(21-47), were genetically engineered onto the N-terminus of the **recombinant** protein and used as an affinity tag for detection and purification The resulting pSec-preS1-E1t310, pSec-preS1-E1t325, and pSec- preS1-E1t340 were transiently expressed in the HeLa cells and antigenicity, secretory efficiency, and glycosylation type of the **recombinant** E1 proteins were compared. All of the three **recombinant** proteins could be detected by both preS1 monoclonal antibody and E1 polyclonal antiserum. The expression products were secreted and highly mannose-type glycosylated, with S1E1t325 being secreted, indicating the influence of the hydrophobic regions on the secretion of the E1 protein. Three CHO cell lines expressing the proteins, S1E1t310, S1E1t325, and S1E1t340, were established and CHO/pSecS1E1t325 was chosen for further study. The secreted S1E1t325

could be enriched from cell culture medium by the preS1 antibody-coupled Sepharose. The glycosylation anal. indicated the lack of complex glycogen even after the E1 was secreted via Golgi complexes. The established stable cell lines and anti-preS1 affinity method could be utilized to enrich and purify the **HCV** E1 expressed in mammalian cells, and may be used for further characterization of this protein.

L14 ANSWER 5 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2000:546735 CAPLUS
DOCUMENT NUMBER: 133:295002
TITLE: Characterization of Modified Hepatitis C Virus E2 Proteins Expressed on the Cell Surface
AUTHOR(S): Forns, Xavier; Allander, Tobias; Rohwer-Nutter, Patricia; Bukh, Jens
CORPORATE SOURCE: Hepatitis Viruses Section, National Institutes of Health, Bethesda, MD, 20892, USA
SOURCE: Virology (2000), 274(1), 75-85
CODEN: VIRLAX; ISSN: 0042-6822
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The **envelope** proteins of hepatitis C virus (**HCV**) are the likely targets of neutralizing antibodies and their mol. and functional characterization is relevant for vaccine development. We previously showed that surface-expressed E2 is a better immunogen than intracellular E2 and, therefore, we were interested in exploring more efficient ways to present E2 protein on the cell surface. We found that E2 targeted to the cell surface by replacement of its transmembrane domain did not bring E1 to the surface although E1 could be expressed independently on the cell surface if its transmembrane domain was similarly replaced. FACS anal. suggested that E2 expressed on the cell surface acquired its native conformation more efficiently when **truncated** at aa 661 than when **truncated** at aa 715. The shorter form of **truncated** E2 better retained the ability to bind the second extracellular loop (EC2) of CD81, the putative **HCV** receptor. Interestingly, deletion of the hypervariable region 1 (HVR1) did not perceptibly alter E2 structure; cell-surface forms of E2 lacking the HVR1 remained reactive with conformation-sensitive MAbs and were able to bind **recombinant** EC2 of CD81. (c) 2000 Academic Press.
REFERENCE COUNT: 37 THERE ARE 37 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 6 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2000:494559 CAPLUS
DOCUMENT NUMBER: 133:221325
TITLE: Evaluation of hepatitis C virus glycoprotein E2 for vaccine design: an endoplasmic reticulum-retained **recombinant** protein is superior to secreted **recombinant** protein and DNA-based vaccine candidates
AUTHOR(S): Heile, Jens M.; Fong, Yiu-Lian; Rosa, Domenico; Berger, Kim; Saletti, Giulietta; Campagnoli, Susanna; Bensi, Giuliano; Capo, Sabrina; Coates, Steve; Crawford, Kevin; Dong, Christine; Wininger, Mark; Baker, Gary; Cousens, Larry; Chien, David; Ng, Philip; Archangel, Phillip; Grandi, Guido; Houghton, Michael; Abrignani, Sergio
CORPORATE SOURCE: IRIS Research Center, Siena, 53100, Italy
SOURCE: Journal of Virology (2000), 74(15), 6885-6892
CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Hepatitis C virus (**HCV**) is the leading causative agent of blood-borne chronic hepatitis and is the target of intensive vaccine research. The virus genome encodes a number of structural and nonstructural antigens which could be used in a subunit vaccine. The **HCV** **envelope** glycoprotein E2 has recently been shown to bind CD81 on human cells and therefore is a prime candidate for inclusion in any such

vaccine. The expts. presented here assessed the optimal form of **HCV** E2 antigen from the perspective of antibody generation. The quality of **recombinant** E2 protein was evaluated by both the capacity to bind its putative receptor CD81 on human cells and the ability to elicit antibodies that inhibited this binding (NOB antibodies). We show that **truncated** E2 proteins expressed in mammalian cells bind with high efficiency to human cells and elicit NOB antibodies in guinea pigs only when purified from the core-glycosylated intracellular fraction, whereas the complex-glycosylated secreted fraction does not bind and elicits no NOB antibodies. We also show that carbohydrate moieties are not necessary for E2 binding to human cells and that only the monomeric nonaggregated fraction can bind to CD81. Moreover, comparing **recombinant** intracellular E2 protein to several E2-encoding DNA vaccines in mice, we found that protein immunization is superior to DNA in both the quantity and quality of the antibody response elicited. Together, our data suggest that to elicit antibodies aimed at blocking **HCV** binding to CD81 on human cells, the antigen of choice is a mammalian cell-expressed, monomeric E2 protein purified from the intracellular fraction.

REFERENCE COUNT: 52 THERE ARE 52 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 7 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1999:290054 CAPLUS
DOCUMENT NUMBER: 131:113495
TITLE: Comparison of secretion of a hepatitis C virus glycoprotein in Saccharomyces cerevisiae and Kluyveromyces lactis
AUTHOR(S): Mustilli, Anna Chiara; Izzo, Emanuela; Houghton, Michael; Galeotti, Cesira L.
CORPORATE SOURCE: Chiron Vaccines, I.R.I.S., Siena, 53100, Italy
SOURCE: Research in Microbiology (1999), 150(3), 179-187
CODEN: RMCREW; ISSN: 0923-2508
PUBLISHER: Editions Scientifiques et Medicales Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A C-terminally **truncated** form of the hepatitis C virus ( **HCV**) putative **envelope** glycoprotein E2 was expressed in two yeast species, Saccharomyces cerevisiae and Kluyveromyces lactis, using a yeast signal peptide sequence to direct the viral glycoprotein to the endoplasmic reticulum (ER) pathway of secretion. Characterization of secreted E2 showed that the protein is endoglycosidase-H-sensitive in both yeasts. Moreover, in vivo inhibition of glycosylation with tunicamycin prevented secretion of E2 and showed that, of its 11 putative N-linked glycosylation sites, at least eight were core-glycosylated. Anal. of the heterologous glycoprotein by SDS-PAGE under nonreducing conditions and by gel filtration demonstrated the formation of multiple disulfides, which resulted in secretion of heterogeneous aggregates with an average mol. mass of 770-1000 kDa in both yeasts. However, variations were observed in the binding of the glycoprotein secreted by the two yeasts to a mannose-specific lectin, and also in its reactivity with anti-E2-specific antibodies. This denotes differences between the two yeasts in folding and/or modification of the E2 glycoprotein.

REFERENCE COUNT: 32 THERE ARE 32 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 8 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1998:810677 CAPLUS
DOCUMENT NUMBER: 130:166917
TITLE: Identification of a domain containing B-cell epitopes in hepatitis C virus E2 glycoprotein by using mouse monoclonal antibodies
AUTHOR(S): Woo Lee, Jae; Kim, Kwang-Mi; Jung, Seung-Hye; Lee, Ki Jeong; Choi, Eung-Chil; Sung, Young-Chul; Kang, Chang-Yuil
CORPORATE SOURCE: Laboratory of Immunology, College of Pharmacy, Seoul National University, Seoul, 151-742, S. Korea
SOURCE: Journal of Virology (1999), 73(1), 11-18
CODEN: JOVIAM; ISSN: 0022-538X

PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Evidence from clin. and exptl. studies of human and chimpanzees suggests that hepatitis C virus (**HCV**) **envelope** glycoprotein E2 is a key antigen for developing a vaccine against **HCV** infection. To identify B-cell epitopes in **HCV** E2, six murine monoclonal antibodies (MAbs), CET-1 to -6, specific for **HCV** E2 protein were generated by using **recombinant** proteins containing E2t (a C-terminally **truncated** domain of **HCV** E2 [amino acids 386 to 693] fused to human growth hormone and glycoprotein D). We tested whether **HCV**-infected sera were able to inhibit the binding of CET MAbs to the former fusion protein. Inhibitory activity was observed in most sera tested, which indicated that CET-1 to -6 were similar to anti-E2 antibodies in human sera with respect to the epitope specificity. The spacial relationship of epitopes on E2 recognized by CET MAbs was determined by surface plasmon resonance anal. and competitive ELISA. The data indicated that three overlapping epitopes were recognized by CET-1 to -6. For mapping the epitopes recognized by CET MAbs, we analyzed the reactivities of CET MAbs to six **truncated** forms and two chimeric forms of **recombinant** E2 proteins. The data suggest that the epitopes recognized by CET-1 to -6 are located in a small domain of E2 spanning amino acid residues 528 to 546.
REFERENCE COUNT: 46 THERE ARE 46 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 9 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1998:592014 CAPLUS
DOCUMENT NUMBER: 129:301407
TITLE: Hepatitis C virus **envelope** DNA-based immunization elicits humoral and cellular immune responses
AUTHOR(S): Lee, Seung Woo; Cho, Jae Ho; Lee, Ki Jeong; Sung, Young Chul
CORPORATE SOURCE: Department of Life Science, Center for Biofunctional Molecules, School of Environmental Engineering, Pohang University of Science and Technology, Pohang, 790-784, S. Korea
SOURCE: Molecules and Cells (1998), 8(4), 444-451
CODEN: MOCEEK; ISSN: 1016-8478
PUBLISHER: Springer-Verlag Singapore Pte. Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The vaccine development for hepatitis C virus (**HCV**) is highly urgent to prevent non A and non B hepatitis. It was recently shown that the **HCV envelope** proteins appeared to the key viral antigens to induce protective immunity. To generate immune responses to the **HCV envelope** proteins on the DNA-based immunization, various **envelope** gene-containing plasmids were constructed. For efficient expression and secretion of **envelope** proteins, the signal sequence of each **envelope** protein was replaced with either herpes simplex virus type-1 (HSV-1) gD or signal sequence of gD and **truncated** C-terminal hydrophobic regions of **envelope** proteins. The i.m. injection of these plasmids generated a significant level of antibody titers to the E1 and E2 proteins, which maximally reached 850 and 25,000 resp. The secreted form of each **envelope** protein and the fusion of the highly immunogenic gD proteins were shown to have no significant effect on generating immune responses to the **envelope** proteins. In addition, immunized rats appeared to generate antibodies directed to the homologous HVR-1 peptide. Splenic lymphocytes from immunized rats were shown to induce significant T-cell proliferative responses with the stimulation of **recombinant** E1 and E2 proteins. Our results demonstrated that the **HCV envelope**-DNA based immunization could elicit both humoral and cellular immune responses.
REFERENCE COUNT: 46 THERE ARE 46 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 10 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1998:313394 CAPLUS
DOCUMENT NUMBER: 129:107767
TITLE: Isolation and characterization of human monoclonal antibodies against hepatitis C virus **envelope** glycoproteins
AUTHOR(S): Da Silva Cardoso, Marcia; Siemoneit, Karl; Sturm, Daniela; Krone, Christoph; Moradpour, Darius; Kubanek, Bernhard
CORPORATE SOURCE: Blood Transfusion Service of Baden-Wurttemberg and Department of Transfusion Medicine, University of Ulm, Germany
SOURCE: Journal of Medical Virology (1998), 55(1), 28-34
CODEN: JMVIDB; ISSN: 0146-6615
PUBLISHER: Wiley-Liss, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The isolation and characterization of human monoclonal antibodies (humAbs) against the hepatitis C virus (**HCV**) glycoproteins E1 and E2 are described. B-cells from blood donors with anti-**HCV** were transformed with Epstein-Barr virus. The supernatants of the resulting lymphoblastoid clones were screened by ELISA with an extract of cells infected with a **recombinant** vaccinia virus RMPA95 expressing the **envelope** proteins E1 and E2 of an **HCV** genotype 1a virus (H strain). Pos. clones were fused to the heteromyeloma cell line K6H6/B5. Fifteen heterohybridoma cell lines have been established. The specificity of the isolated humAbs was determined both by ELISA and Western blot assays. Several **recombinant** exts. expressing either the E1 or E2 protein or **truncated** forms were used in an attempt to map the epitopes on the viral glycoproteins. Some of the humAbs were used successfully for immunofluorescence investigation of transfected cells. Seven specific anti-E2 humAbs, which react with the **envelope** protein 2 of genotype 1a and 1b isolates, were characterized.
REFERENCE COUNT: 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 11 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1997:775256 CAPLUS
DOCUMENT NUMBER: 128:72872
TITLE: Hepatitis C virus E2 protein purified from mammalian cells is frequently recognized by E2-specific antibodies in patient sera
AUTHOR(S): Lee, Ki Jeong; Suh, Young-Ah; Cho, Young Gyu; Cho, Young Shik; Ha, Gun Woo; Chung, Kwang-Hoe; Hwang, Jae Hoon; Yun, Young Dae; Lee, Dong Soon; Kim, Chang Min; Sung, Young-Chul
CORPORATE SOURCE: Department of Life Science, Center for Biofunctional Molecules, School of Environmental Engineering, Pohang University of Science and Technology, Pohang, 790-784, S. Korea
SOURCE: Journal of Biological Chemistry (1997), 272(48), 30040-30046
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The **envelope** protein of hepatitis C virus (**HCV**) is composed of two membrane-associated glycoproteins, E1 and E2. To obtain **HCV** E2 protein as a secretory form at a high level, we constructed a **recombinant** chinese hamster ovary (CHO) cell line expressing a C-terminal **truncated** E2 (E2t) fused to human growth hormone (hGH), CHO/hGHE2t. The hGHE2t fusion protein was purified from the culture supernatant using anti-hGH mAb affinity chromatog. at approx. 80% purity. The purified hGHE2t protein appeared to be assembled into oligomers linked by intermol. disulfide bond(s) when d. gradient centrifugation and SDS-polyacrylamide gel electrophoresis were employed. When the purified fusion protein was used for testing its ability to bind to antibodies specific for **HCV** by ELISA, the protein was recognized by antibodies in sera from 90% of **HCV**-pos. patients.

Treatment of hGHE2t protein by β-mercaptoethanol, but not by heat and SDS, significantly reduced its reactivity to the antibodies of patient sera, suggesting that intermol. and/or intramol. disulfide bonds are important for its ability to recognize its specific antibody and that the E2 protein contains discontinuous antigenic epitope(s).
REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 12 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1997:113448 CAPLUS
DOCUMENT NUMBER: 126:117059
TITLE: Method for detection of antibody to hepatitis C virus second **envelope** glycoprotein
INVENTOR(S): Okasinski, Gregory F.; Schaefer, Verlyn G.; Suhar, Thomas S.; Lesniewski, Richard R.; Scheffel, James W.
PATENT ASSIGNEE(S): Abbott Laboratories, USA
SOURCE: PCT Int. Appl., 34 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 9641196 | A1 | 19961219 | WO 1996-US8536 | 19960604 |
| W: CA, JP | | | | |
| RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE | | | | |
| CA 2223277 | AA | 19961219 | CA 1996-2223277 | 19960604 |
| EP 836708 | A1 | 19980422 | EP 1996-917969 | 19960604 |
| R: AT, BE, CH, DE, ES, FR, GB, IT, LI, NL | | | | |
| JP 11507129 | T2 | 19990622 | JP 1996-501105 | 19960604 |
| PRIORITY APPLN. INFO.: | | | US 1995-481018 A | 19950607 |
| | | | WO 1996-US8536 W | 19960604 |

AB A method for detecting antibody to **HCV** in a test sample. The method includes utilizing a **recombinant** protein that is the expression product of mammalian cells transformed by a heterologous expression vector comprising a DNA sequencing encoding an E2 **truncated** protein. Test kits which include this **recombinant** protein also are provided.

L14 ANSWER 13 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1996:698698 CAPLUS
DOCUMENT NUMBER: 126:6277
TITLE: Expression of **HCV envelope** proteins and the serological utility of the anti-E2 immune response
AUTHOR(S): Lesniewski, Richard R.; Watanabe, Shinichi; Devare, Sushil G.
CORPORATE SOURCE: Hepatitis Research and Development, Abbott Laboratories, Abbott Park, IL, 60064, USA
SOURCE: Proceedings of the International Symposium of the Princess Takamatsu Cancer Research Fund (1995), Volume Date 1994, 25th(Hepatitis C Virus and Its Involvement in the Development of Hepatocellular Carcinoma), 129-137
CODEN: PPTCBY
PUBLISHER: Princeton Scientific
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The 5' end of the hepatitis C virus (**HCV**) genome encodes structural proteins of the virion. The first gene encodes a highly basic core protein. Immediately downstream of the core gene are regions which encode the **envelope** proteins (E1 and E2) of the virus. Artificial expression and secretion of immunol. active **envelope** proteins have proven to be a substantial challenge due to the high degree of glycosylation and the existence of certain hydrophobic domains contained within these sequences. Bacterial cell expression of **recombinant HCV envelope** proteins results in

products that are not glycosylated and are poorly immunogenic. Emphasis has shifted to the use of mammalian cell lines (human embryonic kidney [HEK] and Chinese hamster ovary [CHO] cells) for the expression of glycosylated, immunol. active **envelope** proteins. Using HEK cells, E1 is expressed intracellularly but is not secreted from the cells. When E1 is cloned in fusion with a C-terminal **truncated** E2 protein, both proteins are detected intracellularly; however, only E2 is secreted. When the E1/E2 processing site is interrupted by constructing deletion mutants, the unprocessed E1/E2 fusion protein can be secreted from the cells. Quantifiable expression and secretion of a **truncated** E2 protein is now possible using CHO cells and SV40-based vectors. The **HCV** E2 glycoprotein expressed from CHO cells is highly antigenic; a strong humoral response to this antigen develops in persons infected with **HCV**. Antibodies to E2 are found in 95% of patients with detectable **HCV** RNA in their sera. The presence of antibodies to E2 is not indicative of viral clearance and therefore the role these antibodies play in protective immunity, if any, is unclear.

L14 ANSWER 14 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1996:260624 CAPLUS
DOCUMENT NUMBER: 124:312065
TITLE: Processing of the E1 glycoprotein of hepatitis C virus expressed in mammalian cells
AUTHOR(S): Fournillier-Jacob, Anne; Cahour, Annie; Escriou, Nicolas; Girard, Marc; Wychowski, Czeslaw
CORPORATE SOURCE: Institut Pasteur, Unite Virologie Moleculaire, Paris, 75724, Fr.
SOURCE: Journal of General Virology (1996), 77(5), 1055-64
CODEN: JGVIAY; ISSN: 0022-1317
PUBLISHER: Society for General Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The structural part of the hepatitis C virus (**HCV**) genome encodes a capsid protein, C, and two **envelope** glycoproteins, E1 and E2, released from the virus polyprotein precursor by signalase(s) cleavage(s). The processing of E1 was investigated by infecting simian cells with **recombinant** vaccinia viruses expressing parts of the **HCV** structural proteins. When the predicted E1 sequence was expressed alone (amino acid residues 174-370 of the polyprotein) or with the capsid protein gene (residues 1-370), it showed an apparent mol. mass of 35 kDa as measured by SDS-PAGE anal. However, when E1 was expressed as part of a **truncated** C-E1-**truncated** E2 polypeptide (residues 132-383), the processed E1 product had the expected apparent mol. mass of 31 kDa, suggesting that flanking sequences are necessary for the generation of the mature 31 kDa E1 form. The N-terminal sequence of the two E1 forms was found to be the same. Anal. of the glycosylation pattern showed that, in both species, only four of the five potential N-linked glycosylation sites were recognized, indicating that glycosylation was not involved in the mol. mass difference. We showed that expression of E1 with or without the hydrophobic stretch of amino acids residues 371-383, defined as the E2 signal sequence, may be responsible for the difference in electrophoretic mobility of the two E1 species. In vitro translation assays and site-directed mutagenesis expts. suggest that this sequence remains part of the 31 kDa E1 mature protein.

=> D L14 IBIB abs 15

L14 ANSWER 15 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1994:698202 CAPLUS
DOCUMENT NUMBER: 121:298202
TITLE: Processing of E1 and E2 glycoproteins of hepatitis C virus expressed in mammalian and insect cells
AUTHOR(S): Matsuura, Yoshiharu; Suzuki, Tetsuro; Suzuki, Ryosuke; Sato, Mitsuru; Aizaki, Hideki; Saito, Izumu; Miyamura, Tatsuo
CORPORATE SOURCE: Dep. Virology II, Natl. Inst. Health, Tokyo, 162, Japan

SOURCE: Virology (1994), 205(1), 141-50
CODEN: VIRLAX; ISSN: 0042-6822
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Processing of the **envelope** glycoproteins (E1 and E2) of hepatitis C virus (**HCV**) was investigated by using cDNA clones covering the structural and part of the nonstructural (NS) protein regions. The cDNA clones expressed in mammalian and insect cells were immunopptd. by serum of a hepatitis C patient and by monoclonal and polyclonal antibodies riased against the **recombinant** proteins expressed in insect cells or Escherichia coli. The E2 protein expressed in both insect and mammalian cells was a glycoprotein of 60 kDa (gp60) and removal of the sugar residues by N-glycanase yielded 38- and 40-kDa proteins. Pulse-chase expts. revealed that efficient expression and processing of the **envelope** proteins required coexpression with the flanking core and NS2 proteins. Not only E1 and E2 proteins but also NS2 and NS3 proteins were copptd. by anti-E1 or anti-E2 monoclonal antibody in the cells infected with the **recombinant** baculovirus expressing structural and NS proteins (NS2 and NS3), while only the NS3 protein was precipitated by anti-NS3 antibody. The association of E1 and E2 proteins was not influenced by the presence of a reducing agent and was still observed in the cells coinfected with the deletion mutants lacking both internal and C-terminal hydrophobic regions of each protein. Furthermore, the **truncated** forms of the E1 and E2 proteins were secreted into the culture supernatant and some of them were still associated with each other.

=> D L13 IBIB ABS

L13 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2001:338732 CAPLUS
DOCUMENT NUMBER: 134:352270
TITLE: Fusion proteins containing antigenic ectodomain of measles virus hemagglutin protein and viral targeting peptides and its use as vaccines
INVENTOR(S): Petrik, Juraj
PATENT ASSIGNEE(S): UK
SOURCE: PCT Int. Appl., 27 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2001032893 | A1 | 20010510 | WO 2000-GB4191 | 20001101 |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| CA 2389339 | AA | 20010510 | CA 2000-2389339 | 20001101 |
| AU 2001011560 | A5 | 20010514 | AU 2001-11560 | 20001101 |
| EP 1226256 | A1 | 20020731 | EP 2000-973003 | 20001101 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR | | | | |
| JP 2003514518 | T2 | 20030422 | JP 2001-535575 | 20001101 |
| PRIORITY APPLN. INFO.: | | | GB 1999-25966 A | 19991102 |
| | | | WO 2000-GB4191 W | 20001101 |

AB A **recombinant** bifunctional fusion protein comprises a first component which is a **mutated** antigenic ectodomain of measles virus hemagglutin protein (MeaH); and a second component fused thereto which is capable of binding to the surface structure of genetically

variable viruses such as **HCV** or HIV or other therapeutic targets. The MeaH antigenic ectodomain is genetically modified and does not to bind to CD46 receptor or cause hemadsorption or hemagglutination, but retains its antigenicity and is recognized by anti-measles antibodies, thus it serves as booster/carrier antigen. The second component binds to the target and the first component is recognized by anti-measles antibodies present in the majority of the population. Examples of second component for HIV gp120env targeting are provided by screening human expression cDNA library with biotinylated **recombinant** env. The protein may be used therapeutically to treat **HCV** or HIV infection or against other therapeutic targets.

REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> D L12 IBIB ABS 1-5

L12 ANSWER 1 OF 5 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2002:832824 CAPLUS

DOCUMENT NUMBER: 137:351491

TITLE: Production of **recombinant HCV envelope** proteins with expression **vectors** encoding avian lysozyme leader or signal peptide

INVENTOR(S): Sablon, Erwin; Van Broekhoven, Annie; Bosman, Alfons; Depla, Erik; Deschamps, Geert

PATENT ASSIGNEE(S): Innogenetics N.V., Belg.

SOURCE: PCT Int. Appl., 319 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 3

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2002085932 | A2 | 20021031 | WO 2002-BE62 | 20020424 |
| WO 2002085932 | A3 | 20030313 | | |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | | |
| CA 2443740 | AA | 20021031 | CA 2002-2443740 | 20020424 |
| US 2003108561 | A1 | 20030612 | US 2002-128590 | 20020424 |
| US 2003152940 | A1 | 20030814 | US 2002-128587 | 20020424 |
| US 2003211597 | A1 | 20031113 | US 2002-128578 | 20020424 |
| EP 1381671 | A2 | 20040121 | EP 2002-764023 | 20020424 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR | | | | |
| NZ 529019 | A | 20040528 | NZ 2002-529019 | 20020424 |
| JP 2004536582 | T2 | 20041209 | JP 2002-583458 | 20020424 |
| BR 2002009033 | A | 20050111 | BR 2002-9033 | 20020424 |
| ZA 2003008277 | A | 20040708 | ZA 2003-8277 | 20031023 |
| ZA 2003008272 | A | 20050124 | ZA 2003-8272 | 20031023 |
| ZA 2003008274 | A | 20050124 | ZA 2003-8274 | 20031023 |
| BG 108373 | A | 20041230 | BG 2003-108373 | 20031121 |

PRIORITY APPLN. INFO.:

| | | |
|---|---|---|
| EP 2001-870088 | A | 20010424 |
| US 2001-305604P | P | 20010717 |
| WO 2002-BE62 | W | 20020424 |

AB The current invention relates to **vectors** and methods for efficient expression of **HCV envelope** proteins in eukaryotic cells. More particularly said **vectors** comprise the coding sequence for an avian lysozyme signal peptide or a functional

equivalent thereof joined to a **HCV envelope** protein or a part thereof. Said avian lysozyme signal peptide is efficiently removed when the protein comprising said avian lysozyme signal peptide joined to a **HCV envelope** protein or a part thereof is expressed in a eukaryotic cell. Suitable eukaryotic cells include yeast cells such as Saccharomyces or Hansenula cells.

L12 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2001:912910 CAPLUS
DOCUMENT NUMBER: 137:104371
TITLE: Secretory expression of different C-terminal truncated **HCV** E1 proteins in mammalian cells and characterization of the expressed products
AUTHOR(S): Zhu, Jun; Kong, Yuying; Liu, Jing; Zhang, Zuchuan; Wang, Yuan; Li, Guangdi
CORPORATE SOURCE: Institute of Biochemistry and Cell Biology, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, Shanghai, 200031, Peop. Rep. China
SOURCE: Shengwu Huaxue Yu Shengwu Wuli Xuebao (2001), 33(6), 634-640
CODEN: SHWPAU; ISSN: 0582-9879
PUBLISHER: Shanghai Kexue Jishu Chubanshe
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
AB Three fragments of **HCV envelope** 1 (E1) with different C-terminal truncation at aa310, aa325, aa340 were cloned into the mammalian expression **vector** pSecTagB. An epitope in the hepatitis B surface antigen, preS1(21-47), were genetically engineered onto the N-terminus of the **recombinant** protein and used as an affinity tag for detection and purification The resulting pSec-preS1-E1t310, pSec-preS1-E1t325, and pSec- preS1-E1t340 were transiently expressed in the HeLa cells and antigenicity, secretory efficiency, and **glycosylation** type of the **recombinant** E1 proteins were compared. All of the three **recombinant** proteins could be detected by both preS1 monoclonal antibody and E1 polyclonal antiserum. The expression products were secreted and highly mannose-type glycosylated, with S1E1t325 being secreted, indicating the influence of the hydrophobic regions on the secretion of the E1 protein. Three CHO cell lines expressing the proteins, S1E1t310, S1E1t325, and S1E1t340, were established and CHO/pSecS1E1t325 was chosen for further study. The secreted S1E1t325 could be enriched from cell culture medium by the preS1 antibody-coupled Sepharose. The **glycosylation** anal. indicated the lack of complex glycogen even after the E1 was secreted via Golgi complexes. The established stable cell lines and anti-preS1 affinity method could be utilized to enrich and purify the **HCV** E1 expressed in mammalian cells, and may be used for further characterization of this protein.

L12 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1996:698698 CAPLUS
DOCUMENT NUMBER: 126:6277
TITLE: Expression of **HCV envelope** proteins and the serological utility of the anti-E2 immune response
AUTHOR(S): Lesniewski, Richard R.; Watanabe, Shinichi; Devare, Sushil G.
CORPORATE SOURCE: Hepatitis Research and Development, Abbott Laboratories, Abbott Park, IL, 60064, USA
SOURCE: Proceedings of the International Symposium of the Princess Takamatsu Cancer Research Fund (1995), Volume Date 1994, 25th(Hepatitis C Virus and Its Involvement in the Development of Hepatocellular Carcinoma), 129-137
CODEN: PPTCBY
PUBLISHER: Princeton Scientific
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The 5' end of the hepatitis C virus (**HCV**) genome encodes structural proteins of the virion. The first gene encodes a highly basic

core protein. Immediately downstream of the core gene are regions which encode the **envelope** proteins (E1 and E2) of the virus. Artificial expression and secretion of immunol. active **envelope** proteins have proven to be a substantial challenge due to the high degree of **glycosylation** and the existence of certain hydrophobic domains contained within these sequences. Bacterial cell expression of **recombinant HCV envelope** proteins results in products that are not glycosylated and are poorly immunogenic. Emphasis has shifted to the use of mammalian cell lines (human embryonic kidney [HEK] and Chinese hamster ovary [CHO] cells) for the expression of glycosylated, immunol. active **envelope** proteins. Using HEK cells, E1 is expressed intracellularly but is not secreted from the cells. When E1 is cloned in fusion with a C-terminal truncated E2 protein, both proteins are detected intracellularly; however, only E2 is secreted. When the E1/E2 processing site is interrupted by constructing deletion mutants, the unprocessed E1/E2 fusion protein can be secreted from the cells. Quantifiable expression and secretion of a truncated E2 protein is now possible using CHO cells and SV40-based **vectors**. The **HCV** E2 glycoprotein expressed from CHO cells is highly antigenic; a strong humoral response to this antigen develops in persons infected with **HCV**. Antibodies to E2 are found in 95% of patients with detectable **HCV** RNA in their sera. The presence of antibodies to E2 is not indicative of viral clearance and therefore the role these antibodies play in protective immunity, if any, is unclear.

L12 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1996:295079 CAPLUS
DOCUMENT NUMBER: 124:352673
TITLE: **Recombinant** production and purification of hepatitis C virus **envelope** proteins for diagnostic and therapeutic use
INVENTOR(S): Maertens, Geert; Bosman, Fons; De Martynoff, Guy; Buyse, Marie-Ange
PATENT ASSIGNEE(S): Innogenetics N.V., Belg.
SOURCE: PCT Int. Appl., 146 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 6
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 9604385 | A2 | 19960215 | WO 1995-EP3031 | 19950731 |
| WO 9604385 | A3 | 19960307 | | |
| W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TT, UA | | | | |
| RW: KE, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG | | | | |
| CA 2172273 | AA | 19960215 | CA 1995-2172273 | 19950731 |
| AU 9533824 | A1 | 19960304 | AU 1995-33824 | 19950731 |
| AU 708174 | B2 | 19990729 | | |
| EP 721505 | A1 | 19960717 | EP 1995-930434 | 19950731 |
| EP 721505 | B1 | 20020508 | | |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE | | | | |
| JP 09503396 | T2 | 19970408 | JP 1995-506189 | 19950731 |
| BR 9506059 | A | 19971028 | BR 1995-6059 | 19950731 |
| SG 71728 | A1 | 20000418 | SG 1997-3877 | 19950731 |
| AT 217345 | E | 20020515 | AT 1995-930434 | 19950731 |
| EP 1211315 | A1 | 20020605 | EP 2002-3643 | 19950731 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE | | | | |
| PT 721505 | T | 20021031 | PT 1995-930434 | 19950731 |
| ES 2174957 | T3 | 20021116 | ES 1995-930434 | 19950731 |
| US 6150134 | A | 20001121 | US 1996-612973 | 19960311 |
| US 6245503 | B1 | 20010612 | US 1997-927597 | 19970911 |
| US 6890737 | B1 | 20050510 | US 1997-928757 | 19970912 |

| | | | | | |
|---|---|---|---|---|---|
| AU 757962 | B2 | 20030313 | AU 1999-57127 | | 19991029 |
| AU 9957127 | A1 | 20000217 | | | |
| US 2003036110 | A1 | 20030220 | US 2001-899303 | | 20010706 |
| US 2002182706 | A1 | 20021205 | US 2001-973025 | | 20011010 |
| US 2003095980 | A1 | 20030522 | US 2001-995808 | | 20011129 |
| JP 2004222729 | A2 | 20040812 | JP 2004-51709 | | 20040226 |
| US 2004185061 | A1 | 20040923 | US 2004-825219 | | 20040416 |
| PRIORITY APPLN. INFO.: | | | EP 1994-870132 | A | 19940729 |
| | | | EP 1994-EP94870132 | A | 19940729 |
| | | | EP 1995-930434 | A3 | 19950731 |
| | | | JP 1996-506189 | A3 | 19950731 |
| | | | WO 1995-EP3031 | W | 19950731 |
| | | | US 1996-612973 | A3 | 19960311 |
| | | | US 1997-928017 | B3 | 19970911 |
| | | | EP 1998-EP98870142 | A | 19980624 |
| | | | EP 1999-EP99870033 | A | 19990222 |
| | | | WO 1999-EP4342 | W | 19990623 |
| | | | US 1999-355040 | W | 19990723 |
| | | | EP 1999-870225 | A | 19991027 |
| | | | US 1999-795289 | A1 | 19991207 |
| | | | US 2000-304194P | P | 20001201 |
| | | | US 2001-260669P | P | 20010111 |
| | | | US 2001-315768P | P | 20010830 |
| | | | US 2001-973025 | A2 | 20011010 |

AB **Envelope** proteins E1 and E2 of hepatitis C virus (**HCV** ), their **recombinant** production and purification, their fragments and engineered derivs., their antigenic epitope peptides, their monoclonal antibodies, and their use for diagnostic and therapeutic means are provided. A method is described for purifying **recombinant** **HCV** single or specific oligomeric **envelope** proteins, characterized in that upon lysing the transformed host cells to isolate the recombinantly expressed protein a disulfide bond cleavage or reduction step is carried out with a disulfide bond cleavage agent (such as dithiothreitol and/or Empigen BB) and an SH group protecting agent (such as N-ethylmaleimide). Various forms of the E1 and E2 proteins are constructed by standard genetic techniques using vaccinia virus recombination **vectors**; such proteins are specific for various **HCV** genotypes, may delete the hydrophobic region from E1, or remove various **glycosylation** sites; they may also add factor Xa cleavage sites and His6 tags for improved purification Epitope (such as F, G, H, and I) peptides are used to generate monoclonal antibodies and to monitor disease progression in patients. Furthermore, the **HCV** E1 protein and peptides are used for prognosing and monitoring the clin. effectiveness and/or clin. outcome of **HCV** treatment.

L12 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1992:528131 CAPLUS
DOCUMENT NUMBER: 117:128131
TITLE: Hepatitis C virus asialoglycoproteins manufacture for vaccines or immunoassay
INVENTOR(S): Ralston, Robert O.; Marcus, Frank; Thudium, Kent B.; Gervase, Barbara A.; Hall, John A.
PATENT ASSIGNEE(S): Chiron Corp., USA
SOURCE: PCT Int. Appl., 28 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 8
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 9208734 | A1 | 19920529 | WO 1991-US8272 | 19911107 |
| W: AU, CA, CS, FI, HU, JP, NO, PL, RO, SU | | | | |
| RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE | | | | |
| EP 414475 | A1 | 19910227 | EP 1990-309120 | 19900821 |
| EP 414475 | B1 | 19971210 | | |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE | | | | |
| AT 161041 | E | 19971215 | AT 1990-309120 | 19900821 |

| | | | | | |
|---|---|---|---|---|---|
| ES | 2110411 | T3 | 19980216 | ES 1990-309120 | 19900821 |
| CA | 2064705 | AA | 19910226 | CA 1990-2064705 | 19900822 |
| CA | 2064705 | C | 19990406 | | |
| WO | 9102820 | A1 | 19910307 | WO 1990-US4766 | 19900822 |
| | W: AU, CA, JP | | | | |
| AU | 9063449 | A1 | 19910403 | AU 1990-63449 | 19900822 |
| AU | 655156 | B2 | 19941208 | | |
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| JP | 2001314192 | A2 | 20011113 | JP 2001-75114 | 19900822 |
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| | W: AU, BB, BG, BR, CA, FI, GB, HU, JP, KP, KR, LK, MC, MG, MW, NO, PL, RO, SD, SU | | | | |
| | RW: BF, BJ, CF, CG, CM, GA, ML, MR, SN, TD, TG | | | | |
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| AU | 639560 | B2 | 19930729 | | |
| GB | 2257784 | A1 | 19930120 | GB 1992-20480 | 19910329 |
| BR | 9106309 | A | 19930420 | BR 1991-6309 | 19910329 |
| HU | 62706 | A2 | 19930528 | HU 1992-3146 | 19910329 |
| HU | 217025 | B | 19991129 | | |
| JP | 05508219 | T2 | 19931118 | JP 1991-507636 | 19910329 |
| JP | 2733138 | B2 | 19980330 | | |
| RO | 109916 | B1 | 19950728 | RO 1975-92012 | 19910329 |
| PL | 172133 | B1 | 19970829 | PL 1991-296329 | 19910329 |
| RU | 2130969 | C1 | 19990527 | RU 1991-5053084 | 19910329 |
| EP | 450931 | A1 | 19911009 | EP 1991-302910 | 19910403 |
| EP | 450931 | B1 | 19960612 | | |
| | R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE | | | | |
| EP | 693687 | A1 | 19960124 | EP 1995-114016 | 19910403 |
| EP | 693687 | B1 | 19990728 | | |
| | R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE | | | | |
| AT | 139343 | E | 19960615 | AT 1991-302910 | 19910403 |
| ES | 2088465 | T3 | 19960816 | ES 1991-302910 | 19910403 |
| AT | 182684 | E | 19990815 | AT 1995-114016 | 19910403 |
| ES | 2134388 | T3 | 19991001 | ES 1995-114016 | 19910403 |
| CA | 2095521 | AA | 19920509 | CA 1991-2095521 | 19911107 |
| AU | 9190267 | A1 | 19920611 | AU 1991-90267 | 19911107 |
| AU | 668078 | B2 | 19960426 | | |
| EP | 556292 | A1 | 19930825 | EP 1992-900091 | 19911107 |
| EP | 556292 | B1 | 19991229 | | |
| | R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE | | | | |
| JP | 06504431 | T2 | 19940526 | JP 1992-500944 | 19911107 |
| HU | 66063 | A2 | 19940928 | HU 1993-1336 | 19911107 |
| EP | 842947 | A2 | 19980520 | EP 1997-120661 | 19911107 |
| EP | 842947 | A3 | 20011212 | | |
| EP | 842947 | B1 | 20040421 | | |
| | R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE | | | | |
| RU | 2123528 | C1 | 19981220 | RU 1993-43621 | 19911107 |
| PL | 175610 | B1 | 19990129 | PL 1991-300038 | 19911107 |
| AT | 188220 | E | 20000115 | AT 1992-900091 | 19911107 |
| ES | 2139591 | T3 | 20000216 | ES 1992-900091 | 19911107 |
| RO | 115446 | B1 | 20000228 | RO 1993-626 | 19911107 |
| CA | 2203443 | C | 20010828 | CA 1991-2203443 | 19911107 |
| JP | 2001286290 | A2 | 20011016 | JP 2001-59335 | 19911107 |
| CZ | 289006 | B6 | 20011017 | CZ 1993-824 | 19911107 |
| RU | 2175657 | C2 | 20011110 | RU 1997-115378 | 19911107 |
| JP | 2003093081 | A2 | 20030402 | JP 2002-199317 | 19911107 |
| JP | 2003174875 | A2 | 20030624 | JP 2002-353148 | 19911107 |
| EP | 1471073 | A2 | 20041027 | EP 2004-76119 | 19911107 |
| EP | 1471073 | A3 | 20041201 | | |
| | R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE | | | | |
| FI | 106317 | B1 | 20010115 | FI 1992-4349 | 19920928 |
| NO | 9203839 | A | 19921119 | NO 1992-3839 | 19921001 |
| NO | 310241 | B1 | 20010611 | | |
| FI | 107803 | B1 | 20011015 | FI 1993-2025 | 19930505 |
| NO | 9301680 | A | 19930628 | NO 1993-1680 | 19930507 |
| NO | 304380 | B1 | 19981207 | | |
| LV | 10344 | B | 19960220 | LV 1993-4381 | 19930531 |
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| LT | 3808 | B | 19960325 | LT 1993-1747 | 19931230 |

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| US 5968775 | A | 19991019 | US 1995-438435 | 19950510 |
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| FI 9701702 | A | 19970421 | FI 1997-1702 | 19970421 |
| FI 107804 | B1 | 20011015 | | |
| NO 9702213 | A | 19970514 | NO 1997-2213 | 19970514 |
| NO 304381 | B1 | 19981207 | | |
| PT 102022 | B | 20001229 | PT 1997-102022 | 19970626 |
| CZ 289923 | B6 | 20020417 | CZ 1997-2196 | 19970710 |
| JP 11071395 | A2 | 19990316 | JP 1998-103178 | 19980414 |
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| GR 3031361 | T3 | 20000131 | GR 1999-402455 | 19990929 |
| GR 3032771 | T3 | 20000630 | GR 2000-400473 | 20000228 |
| JP 2004049235 | A2 | 20040219 | JP 2003-180211 | 20030624 |

PRIORITY APPLN. INFO.:

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| US 1989-398667 | A | 19890825 |
| US 1990-611419 | A | 19901108 |
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| US 1991-758880 | A | 19910913 |
| US 1987-122714 | B2 | 19871118 |
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| US 1988-161072 | B2 | 19880226 |
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| US 1990-504352 | A | 19900404 |
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| JP 2001-75114 | A3 | 19900822 |
| WO 1990-US4766 | A | 19900822 |
| WO 1991-US2225 | A | 19910329 |
| EP 1991-302910 | A3 | 19910403 |
| CA 1991-2095521 | A3 | 19911107 |
| CZ 1993-824 | A3 | 19911107 |
| EP 1992-900091 | A3 | 19911107 |
| EP 1997-120661 | A3 | 19911107 |
| JP 1992-500944 | A3 | 19911107 |
| JP 1998-103178 | A3 | 19911107 |
| JP 2001-59335 | A3 | 19911107 |
| WO 1991-US8272 | A | 19911107 |
| US 1992-910760 | A3 | 19920707 |
| FI 1993-2025 | A | 19930505 |
| US 1993-97853 | A1 | 19930727 |

AB Two hepatitis C virus (**HCV**) **envelope** proteins (E1 and E2) are manufactured without sialylation. Expression of these genes in lower eukaryotes, or in mammalian cells in which terminal **glycosylation** is blocked, results in proteins similar to native **HCV** glycoproteins. When isolated by mannose-binding GNA (Galanthus nivalus agglutinin) lectin affinity, the E1 and E2 proteins aggregate into virus-like particles. Cells bearing a mannose receptor or asialoglycoprotein receptor are capable of being infected with **HCV** and of supporting culturing of the virus. E1 and E2 were produced in HeLa S3 cells inoculated with **recombinant** Vaccinia virus containing **HCV** gene fragments and purified using a GNA-agarose column.

=> D L11 IBIB ABS 1-5

L11 ANSWER 1 OF 5 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2002:180436 CAPLUS
DOCUMENT NUMBER: 137:227162
TITLE: Cloning and expression of human CD81 major extracellular loop in E. coli and its activity
AUTHOR(S): Zhang, Guojun; Ling, Shigan; Song, Xiaoguo; Zhang, Heqiu; Chen, Kun; Zhu, Cuixia; Xiu, Bingshui

CORPORATE SOURCE: Institute of Basic Medical Sciences, Academy of Military Medical Sciences, Beijing, 100850, Peop. Rep. China
SOURCE: Junshi Yixue Kexueyuan Yuankan (2001), 25(4), 260-264
CODEN: JYKYEL; ISSN: 1000-5501
PUBLISHER: Junshi Yixue Kexueyuan Yuankan Bianjibu
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
AB An expression plasmid for a fusion protein of human CD81 major extracellular loop was constructed and binding activity of its expressed protein with **HCV** E2 was studied. CD81 major extracellular loop sequence was amplified from human peripheral blood lymphocytes by RT-PCR, then inserted into the expression **vector** pBVIL1, and expressed in E. coli. The purified fusion protein was tested for binding activity with E2. CD81-EC2 gene was correctly amplified and inserted into the **vector** as confirmed by sequencing. The preliminary study showed that the **recombinant** CD81/EC2 could bind **truncated** **HCV** E2 (384-661) protein expressed in E. coli. This work proved the way for further study on interactions of CD81 with **HCV** and its E2, and for preparation of anti-EC2 monoclonal antibody.

L11 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2001:912910 CAPLUS
DOCUMENT NUMBER: 137:104371
TITLE: Secretory expression of different C-terminal **truncated** **HCV** E1 proteins in mammalian cells and characterization of the expressed products
AUTHOR(S): Zhu, Jun; Kong, Yuying; Liu, Jing; Zhang, Zuchuan; Wang, Yuan; Li, Guangdi
CORPORATE SOURCE: Institute of Biochemistry and Cell Biology, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, Shanghai, 200031, Peop. Rep. China
SOURCE: Shengwu Huaxue Yu Shengwu Wuli Xuebao (2001), 33(6), 634-640
CODEN: SHWPAU; ISSN: 0582-9879
PUBLISHER: Shanghai Kexue Jishu Chubanshe
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
AB Three fragments of **HCV** **envelope** 1 (E1) with different C-terminal truncation at aa310, aa325, aa340 were cloned into the mammalian expression **vector** pSecTagB. An epitope in the hepatitis B surface antigen, preS1(21-47), were genetically engineered onto the N-terminus of the **recombinant** protein and used as an affinity tag for detection and purification The resulting pSec-preS1-E1t310, pSec-preS1-E1t325, and pSec- preS1-E1t340 were transiently expressed in the HeLa cells and antigenicity, secretory efficiency, and glycosylation type of the **recombinant** E1 proteins were compared. All of the three **recombinant** proteins could be detected by both preS1 monoclonal antibody and E1 polyclonal antiserum. The expression products were secreted and highly mannose-type glycosylated, with S1E1t325 being secreted, indicating the influence of the hydrophobic regions on the secretion of the E1 protein. Three CHO cell lines expressing the proteins, S1E1t310, S1E1t325, and S1E1t340, were established and CHO/pSecS1E1t325 was chosen for further study. The secreted S1E1t325 could be enriched from cell culture medium by the preS1 antibody-coupled Sepharose. The glycosylation anal. indicated the lack of complex glycogen even after the E1 was secreted via Golgi complexes. The established stable cell lines and anti-preS1 affinity method could be utilized to enrich and purify the **HCV** E1 expressed in mammalian cells, and may be used for further characterization of this protein.

L11 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1998:592014 CAPLUS
DOCUMENT NUMBER: 129:301407
TITLE: Hepatitis C virus **envelope** DNA-based immunization elicits humoral and cellular immune responses
AUTHOR(S): Lee, Seung Woo; Cho, Jae Ho; Lee, Ki Jeong; Sung,

Young Chul
CORPORATE SOURCE: Department of Life Science, Center for Biofunctional Molecules, School of Environmental Engineering, Pohang University of Science and Technology, Pohang, 790-784, S. Korea
SOURCE: Molecules and Cells (1998), 8(4), 444-451
CODEN: MOCEEK; ISSN: 1016-8478
PUBLISHER: Springer-Verlag Singapore Pte. Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The vaccine development for hepatitis C virus (**HCV**) is highly urgent to prevent non A and non B hepatitis. It was recently shown that the **HCV envelope** proteins appeared to the key viral antigens to induce protective immunity. To generate immune responses to the **HCV envelope** proteins on the DNA-based immunization, various **envelope** gene-containing plasmids were constructed. For efficient expression and secretion of **envelope** proteins, the signal sequence of each **envelope** protein was replaced with either herpes simplex virus type-1 (HSV-1) gD or signal sequence of gD and **truncated** C-terminal hydrophobic regions of **envelope** proteins. The i.m. injection of these plasmids generated a significant level of antibody titers to the E1 and E2 proteins, which maximally reached 850 and 25,000 resp. The secreted form of each **envelope** protein and the fusion of the highly immunogenic gD proteins were shown to have no significant effect on generating immune responses to the **envelope** proteins. In addition, immunized rats appeared to generate antibodies directed to the homologous HVR-1 peptide. Splenic lymphocytes from immunized rats were shown to induce significant T-cell proliferative responses with the stimulation of **recombinant** E1 and E2 proteins. Our results demonstrated that the **HCV envelope**-DNA based immunization could elicit both humoral and cellular immune responses.

REFERENCE COUNT: 46 THERE ARE 46 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1997:113448 CAPLUS
DOCUMENT NUMBER: 126:117059
TITLE: Method for detection of antibody to hepatitis C virus second **envelope** glycoprotein
INVENTOR(S): Okasinski, Gregory F.; Schaefer, Verlyn G.; Suhar, Thomas S.; Lesniewski, Richard R.; Scheffel, James W.
PATENT ASSIGNEE(S): Abbott Laboratories, USA
SOURCE: PCT Int. Appl., 34 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | | DATE |
|---|---|---|---|---|---|
| WO 9641196 | A1 | 19961219 | WO 1996-US8536 | | 19960604 |
| W: CA, JP | | | | | |
| RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE | | | | | |
| CA 2223277 | AA | 19961219 | CA 1996-2223277 | | 19960604 |
| EP 836708 | A1 | 19980422 | EP 1996-917969 | | 19960604 |
| R: AT, BE, CH, DE, ES, FR, GB, IT, LI, NL | | | | | |
| JP 11507129 | T2 | 19990622 | JP 1996-501105 | | 19960604 |
| PRIORITY APPLN. INFO.: | | | US 1995-481018 | A | 19950607 |
| | | | WO 1996-US8536 | W | 19960604 |

AB A method for detecting antibody to **HCV** in a test sample. The method includes utilizing a **recombinant** protein that is the expression product of mammalian cells transformed by a heterologous expression **vector** comprising a DNA sequencing encoding an E2 **truncated** protein. Test kits which include this **recombinant** protein also are provided.

L11 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1996:698698 CAPLUS
DOCUMENT NUMBER: 126:6277
TITLE: Expression of **HCV envelope** proteins and the serological utility of the anti-E2 immune response
AUTHOR(S): Lesniewski, Richard R.; Watanabe, Shinichi; Devare, Sushil G.
CORPORATE SOURCE: Hepatitis Research and Development, Abbott Laboratories, Abbott Park, IL, 60064, USA
SOURCE: Proceedings of the International Symposium of the Princess Takamatsu Cancer Research Fund (1995), Volume Date 1994, 25th(Hepatitis C Virus and Its Involvement in the Development of Hepatocellular Carcinoma), 129-137
CODEN: PPTCBY
PUBLISHER: Princeton Scientific
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The 5' end of the hepatitis C virus (**HCV**) genome encodes structural proteins of the virion. The first gene encodes a highly basic core protein. Immediately downstream of the core gene are regions which encode the **envelope** proteins (E1 and E2) of the virus. Artificial expression and secretion of immunol. active **envelope** proteins have proven to be a substantial challenge due to the high degree of glycosylation and the existence of certain hydrophobic domains contained within these sequences. Bacterial cell expression of **recombinant HCV envelope** proteins results in products that are not glycosylated and are poorly immunogenic. Emphasis has shifted to the use of mammalian cell lines (human embryonic kidney [HEK] and Chinese hamster ovary [CHO] cells) for the expression of glycosylated, immunol. active **envelope** proteins. Using HEK cells, E1 is expressed intracellularly but is not secreted from the cells. When E1 is cloned in fusion with a C-terminal **truncated** E2 protein, both proteins are detected intracellularly; however, only E2 is secreted. When the E1/E2 processing site is interrupted by constructing deletion mutants, the unprocessed E1/E2 fusion protein can be secreted from the cells. Quantifiable expression and secretion of a **truncated** E2 protein is now possible using CHO cells and SV40-based **vectors**. The **HCV** E2 glycoprotein expressed from CHO cells is highly antigenic; a strong humoral response to this antigen develops in persons infected with **HCV**. Antibodies to E2 are found in 95% of patients with detectable **HCV** RNA in their sera. The presence of antibodies to E2 is not indicative of viral clearance and therefore the role these antibodies play in protective immunity, if any, is unclear.

=> D L9 IBIB ABS 1-9

L9 ANSWER 1 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2004:402741 CAPLUS
DOCUMENT NUMBER: 140:373891
TITLE: **Recombinant** hepatitis C virus E1 and E2 **envelope** proteins for diagnostic and therapeutic use
INVENTOR(S): Maertens, Geert; Bosman, Fons; Buyse, Marie Ange
PATENT ASSIGNEE(S): Belg.
SOURCE: U.S. Pat. Appl. Publ., 162 pp., Cont.-in-part of U.S. Ser. No. 355,040.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 6
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| US 2003118603 | A1 | 20030626 | US 2001-995860 | 20011129 |
| WO 9967285 | A1 | 19991229 | WO 1999-EP4342 | 19990623 |

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

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| US 6635257 | B1 | 20031021 | US 1999-355040 | 19990723 |
| ZA 2000007318 | A | 20030310 | ZA 2000-7318 | 20001208 |
| TR 200202169 | T1 | 20040621 | TR 2002-200202169 | 20020111 |
| ZA 2002007272 | A | 20040213 | ZA 2002-7272 | 20020910 |

PRIORITY APPLN. INFO.:

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|---|---|---|
| EP 1998-870142 | A | 19980624 |
| EP 1999-870033 | A | 19990222 |
| WO 1999-EP4342 | W | 19990623 |
| US 1999-355040 | A2 | 19990723 |
| US 2000-304194P | P | 20001201 |
| US 2001-260669P | P | 20010111 |
| US 2001-315768P | P | 20010830 |

AB The present invention relates to a method for purifying **recombinant HCV** single or specific oligomeric **envelope** proteins selected from the group consisting of E1 and/or E2 and/or E1/E2, characterized in that upon lysing the transformed host cells to isolate the recombinantly expressed protein a disulfide bond cleavage or reduction step is carried out with a disulfide bond cleavage agent. The present invention also relates to a composition isolated by such a method. The present invention also relates to the diagnostic and therapeutic application of these compns. Furthermore, the invention relates to the use of **HCV** E1 protein and peptides for prognosing and monitoring the clin. effectiveness and/or clin. outcome of **HCV** treatment.

L9 ANSWER 2 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN

| | |
|---|---|
| ACCESSION NUMBER: | 2003:756030 CAPLUS |
| DOCUMENT NUMBER: | 139:349007 |
| TITLE: | Lethality in mice infected with **recombinant vaccinia** virus expressing hepatitis C virus core protein |
| AUTHOR(S): | Zhang, Hong |
| CORPORATE SOURCE: | ISIS Pharmaceuticals, Carlsbad, CA, 92008, USA |
| SOURCE: | Hepatobiliary & Pancreatic Diseases International (2003), 2(3), 374-382<br>CODEN: HPDIAJ; ISSN: 1499-3872 |
| PUBLISHER: | First Affiliated Hospital, Zhejiang University School of Medicine |
| DOCUMENT TYPE: | Journal |
| LANGUAGE: | English |

AB OBJECTIVE: To establish a mouse model of **HCV** core expression and investigate the toxicity of **HCV** core protein or the possible pathogenic effects. METHODS: A series of **vaccinia** viral expression **vectors** were engineered to express 5' portion of **HCV** genes including 5' non-translated region (NTR), core protein, and portion of the E1 gene. These **HCV** sequences were fused to a luciferase reporter gene and inserted into a **vaccinia** virus expression **vector** (pSC11) adjacent to the **vaccinia** virus promoter, p7.5. The **recombinant** DNA constructs were packed into infectious **recombinant** chimeric viruses. The expression of **HCV** core protein was examined in cultured cells after infection with these viruses. Death of the infected mice was investigated by specific correlation to the expression of **HCV** core protein and its expression levels. RESULTS: The **recombinant** virus (VNCE-LUA) expressed **HCV** core protein and an **envelope**-luciferase fusion protein in cultured cells. When Balb/c mice were inoculated i.p. with more than 107 pfu per mouse of VNCE-LUA, death occurred immediately. The mortality was dependent on the amount of VNCE-LUA virus inoculated. All mice inoculated with 3 + 108 pfu of VNCE-LUA died within 4 days of infection and 50% of mice inoculated with 3 + 107 pfu of VNCE-LUA died within 7 days of infection. No death

occurred in mice inoculated with 3 + 108 pfu of a control **recombinant vaccinia** virus, which expressed luciferase but not the **HCV** core and **envelope** proteins. Deletion of core sequences from VNCE-LUA rapidly reduced the mortality of infected mice whereas deletion of **envelope** sequence did not. SCID mice infected with VNCE-LUA died 2-3 days after infection, suggesting that the **HCV**-core induced mortality is not dependent on host T- or B-cell responses to core protein. CONCLUSIONS: **HCV** core protein can be lethal to mice when expressed in vivo and this specific lethality is independent of T-cells or B-cells. The findings and model itself provide a useful tool for further investigation on potential pathol. effects as well as the potential toxicity of the **HCV** core protein.

REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 3 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2003:491258 CAPLUS
DOCUMENT NUMBER: 139:67765
TITLE: **Recombinant** hepatitis C virus E1 and E2 **envelope** proteins for diagnostic and therapeutic use
INVENTOR(S): Maertens, Geert; Depla, Erik; Bosman, Fons
PATENT ASSIGNEE(S): Innogenetics N.V., Belg.
SOURCE: PCT Int. Appl., 270 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2003051912 | A2 | 20030626 | WO 2002-EP14480 | 20021218 |
| WO 2003051912 | A3 | 20040304 | | |
| WO 2003051912 | C2 | 20040715 | | |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW | | | | |
| RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | | |
| CA 2468690 | AA | 20030626 | CA 2002-2468690 | 20021218 |
| US 2004126395 | A1 | 20040701 | US 2002-321798 | 20021218 |
| EP 1461080 | A2 | 20040929 | EP 2002-796675 | 20021218 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, SK | | | | |
| BR 2002015081 | A | 20041019 | BR 2002-15081 | 20021218 |
| NZ 533396 | A | 20050429 | NZ 2002-533396 | 20021218 |
| JP 2005516939 | T2 | 20050609 | JP 2003-552792 | 20021218 |

PRIORITY APPLN. INFO.:

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| US 2002-418358P | P | 20021016 |
| WO 2002-EP14480 | W | 20021218 |

AB The present invention relates to a method for purifying **recombinant HCV** single or specific oligomeric **envelope** proteins selected from the group consisting of E1 and/or E2 and/or E1/E2, characterized in that upon lysing the transformed host cells to isolate the recombinantly expressed protein a disulfide bond cleavage or reduction step is carried out with a disulfide bond cleavage agent. The present invention also relates to a composition isolated by such a method. The present invention also relates to the diagnostic and therapeutic application of these compns. Furthermore, the invention relates to the use of **HCV** E1 protein and peptides for prognosing and monitoring the clin. effectiveness and/or clin. outcome of **HCV** treatment.

L9 ANSWER 4 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2000:467550 CAPLUS
DOCUMENT NUMBER: 133:236484
TITLE: Induction of hepatitis C virus-specific cytotoxic T lymphocytes in mice by an intrahepatic inoculation with an expression plasmid
AUTHOR(S): Kamei, Akira; Tamaki, Shigenori; Taniyama, Hiroyuki; Takamura, Shiki; Nishimura, Yuki; Kagawa, Yumiko; Uno-Furuta, Satori; Kaito, Masahiko; Kim, Gisen; Toda, Masaaki; Matsuura, Yoshiharu; Miyamura, Tatsuo; Adachi, Yukihiko; Yasutomi, Yasuhiro
CORPORATE SOURCE: Department of Bioregulation, Mie University School of Medicine, Mie, 514-8507, Japan
SOURCE: Virology (2000), 273(1), 120-126
CODEN: VIRLAX; ISSN: 0042-6822
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The authors assessed the possibility of intrahepatic inoculation with a plasmid encoding hepatitis C virus (**HCV**) proteins to elicit **HCV**-specific cytotoxic T lymphocytes (CTL) in mice as a conventional animal model of **HCV** infection. BALB/c mice were intrahepatically or i.m. inoculated with an expression plasmid DNA encoding **HCV** structural proteins under the control of the elongation factor 1-α promoter. Expressions of **HCV**-core protein and **envelope** proteins (E1 and E2) in hepatocytes were detected immunohistochem. 6 days after inoculation. CTL responses were examined using target cells either pulsed with a specific peptide or infected with a **recombinant vaccinia** virus expressing **HCV** structural protein. Both intrahepatically and i.m. DNA-inoculated mice developed CD8+, MHC class I-restricted CTL responses that recognized the peptide pulsed as well as **HCV** proteins expressing target cells. These studies demonstrated the usefulness of a murine model of **HCV** infection induced by direct intrahepatic DNA inoculation for understanding the immunopathogenic mechanisms in **HCV** infection. (c) 2000 Academic Press.
REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 5 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1998:205500 CAPLUS
DOCUMENT NUMBER: 128:290843
TITLE: Expression of structural proteins of hepatitis C virus (**HCV**) in mammalian cells
AUTHOR(S): Li, Yingchun; Li, Guangdi; Kong, Yuying; Wang, Yuan; Wang, Yu; Wen, Yumei
CORPORATE SOURCE: Shanghai Inst. Biochemistry, Chinese Academy Sciences, Shanghai, 200031, Peop. Rep. China
SOURCE: Science in China, Series C: Life Sciences (1998), 41(1), 47-55
CODEN: SCCLFO; ISSN: 1006-9305
PUBLISHER: Science in China Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The **vaccinia** viral **vector** containing T7 promoter was used to construct the expression plasmids carrying **HCV** structural genes of C, E1 and E2/NS1. These genes were transiently expressed in mammalian cells in the presence of the T7 RNA polymerase which was provided by the **recombinant vaccinia** virus vTT7. Expression of mature core protein, **envelope** protein E1 and E2 was detected by Western blot using **HCV** patient sera as the primary antibodies. It was found that the sera from different **HCV** patients reacted differently with the expressed products, so did the sera collected at different times from the same patient, from whom the **HCV** structural genes were isolated. Among six mammalian cell lines, Vero and HeLa were the most suitable for the expression of C, E1 and E2. The **recombinant vaccinia** viruses have been constructed to constantly produce the C, E1 and E2 proteins for further research.

REFERENCE COUNT: 12 THERE ARE 12 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1996:295079 CAPLUS
DOCUMENT NUMBER: 124:352673
TITLE: **Recombinant** production and purification of hepatitis C virus **envelope** proteins for diagnostic and therapeutic use
INVENTOR(S): Maertens, Geert; Bosman, Fons; De Martynoff, Guy; Buyse, Marie-Ange
PATENT ASSIGNEE(S): Innogenetics N.V., Belg.
SOURCE: PCT Int. Appl., 146 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 6
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 9604385 | A2 | 19960215 | WO 1995-EP3031 | 19950731 |
| WO 9604385 | A3 | 19960307 | | |
| W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TT, UA | | | | |
| RW: KE, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG | | | | |
| CA 2172273 | AA | 19960215 | CA 1995-2172273 | 19950731 |
| AU 9533824 | A1 | 19960304 | AU 1995-33824 | 19950731 |
| AU 708174 | B2 | 19990729 | | |
| EP 721505 | A1 | 19960717 | EP 1995-930434 | 19950731 |
| EP 721505 | B1 | 20020508 | | |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE | | | | |
| JP 09503396 | T2 | 19970408 | JP 1995-506189 | 19950731 |
| BR 9506059 | A | 19971028 | BR 1995-6059 | 19950731 |
| SG 71728 | A1 | 20000418 | SG 1997-3877 | 19950731 |
| AT 217345 | E | 20020515 | AT 1995-930434 | 19950731 |
| EP 1211315 | A1 | 20020605 | EP 2002-3643 | 19950731 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE | | | | |
| PT 721505 | T | 20021031 | PT 1995-930434 | 19950731 |
| ES 2174957 | T3 | 20021116 | ES 1995-930434 | 19950731 |
| US 6150134 | A | 20001121 | US 1996-612973 | 19960311 |
| US 6245503 | B1 | 20010612 | US 1997-927597 | 19970911 |
| US 6890737 | B1 | 20050510 | US 1997-928757 | 19970912 |
| AU 757962 | B2 | 20030313 | AU 1999-57127 | 19991029 |
| AU 9957127 | A1 | 20000217 | | |
| US 2003036110 | A1 | 20030220 | US 2001-899303 | 20010706 |
| US 2002182706 | A1 | 20021205 | US 2001-973025 | 20011010 |
| US 2003095980 | A1 | 20030522 | US 2001-995808 | 20011129 |
| JP 2004222729 | A2 | 20040812 | JP 2004-51709 | 20040226 |
| US 2004185061 | A1 | 20040923 | US 2004-825219 | 20040416 |

| PRIORITY APPLN. INFO.: | | |
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| EP 1994-870132 | A | 19940729 |
| EP 1994-EP94870132 | A | 19940729 |
| EP 1995-930434 | A3 | 19950731 |
| JP 1996-506189 | A3 | 19950731 |
| WO 1995-EP3031 | W | 19950731 |
| US 1996-612973 | A3 | 19960311 |
| US 1997-928017 | B3 | 19970911 |
| EP 1998-EP98870142 | A | 19980624 |
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| WO 1999-EP4342 | W | 19990623 |
| US 1999-355040 | W | 19990723 |
| EP 1999-870225 | A | 19991027 |
| US 1999-795289 | A1 | 19991207 |
| US 2000-304194P | P | 20001201 |
| US 2001-260669P | P | 20010111 |

US 2001-315768P P 20010830
US 2001-973025 A2 20011010

AB **Envelope** proteins E1 and E2 of hepatitis C virus (**HCV**), their **recombinant** production and purification, their fragments and engineered derivs., their antigenic epitope peptides, their monoclonal antibodies, and their use for diagnostic and therapeutic means are provided. A method is described for purifying **recombinant** **HCV** single or specific oligomeric **envelope** proteins, characterized in that upon lysing the transformed host cells to isolate the recombinantly expressed protein a disulfide bond cleavage or reduction step is carried out with a disulfide bond cleavage agent (such as dithiothreitol and/or Empigen BB) and an SH group protecting agent (such as N-ethylmaleimide). Various forms of the E1 and E2 proteins are constructed by standard genetic techniques using **vaccinia** virus recombination **vectors**; such proteins are specific for various **HCV** genotypes, may delete the hydrophobic region from E1, or remove various glycosylation sites; they may also add factor Xa cleavage sites and His6 tags for improved purification Epitope (such as F, G, H, and I) peptides are used to generate monoclonal antibodies and to monitor disease progression in patients. Furthermore, the **HCV** E1 protein and peptides are used for prognosing and monitoring the clin. effectiveness and/or clin. outcome of **HCV** treatment.

L9 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1994:602124 CAPLUS
DOCUMENT NUMBER: 121:202124
TITLE: Formation and intracellular localization of hepatitis C virus **envelope** glycoprotein complexes expressed by **recombinant vaccinia** and Sindbis viruses
AUTHOR(S): Dubuisson, Jean; Hsu, Henry H.; Cheung, Ramsey C.; Greenberg, Harry B.; Russell, David G.; Rice, Charles M.
CORPORATE SOURCE: Sch. Med., Washington Univ., St. Louis, MO, 63110-1093, USA
SOURCE: Journal of Virology (1994), 68(10), 6147-60
CODEN: JOVIAM; ISSN: 0022-538X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Hepatitis C virus (**HCV**) encodes two putative virion glycoproteins (E1 and E2) which are released from the polyprotein by signal peptidase cleavage. In this report, the authors have characterized the complexes formed between E1 and E2 (called E1E2) for two different **HCV** strains (H and BK) and studied their intracellular localization. **Vaccinia** virus and Sindbis virus **vectors** were used to express the **HCV** structural proteins in three different cell lines (HepG2, BHK-21, and PK-15). The kinetics of association between E1 and E2, as studied by pulse-chase anal. and copptn. of E2 with an anti-E1 monoclonal antibody, indicated that formation of stable E1E2 complexes is slow. The times required for half-maximal association between E1 and E2 were 60 to 85 min for the H strain and more than 165 min for the BK strain. In the presence of nonionic detergents, two forms of E1E2 complexes were detected. The predominant form was a heterodimer of E1 and E2 stabilized by noncovalent interactions. A minor fraction consisted of heterogeneous disulfide-linked aggregates, which most likely represent misfolded complexes. Posttranslational processing and localization of the **HCV** glycoproteins were examined by acquisition of endoglycosidase H resistance, subcellular fractionation, immunofluorescence, cell surface immunostaining, and immunoelectron microscopy. **HCV** glycoproteins containing complex N-linked glycans were not observed, and the proteins were not detected at the cell surface. Rather, the proteins localized predominantly to the endoplasmic reticular network, suggesting that some mechanism exists for their retention in this compartment.

L9 ANSWER 8 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1994:4188 CAPLUS
DOCUMENT NUMBER: 120:4188
TITLE: Characterization of hepatitis C virus **envelope** glycoprotein complexes expressed by

**recombinant vaccinia** viruses

AUTHOR(S): Ralston, Robert; Thudium, Kent; Berger, Kim; Kuo, Carol; Gervase, Barbara; Hall, John; Selby, Mark; Kuo, George; Houghton, Michael; Choo, Qui Lim

CORPORATE SOURCE: Chiron Corp., Emeryville, CA, 94608, USA

SOURCE: Journal of Virology (1993), 67(11), 6753-61
CODEN: JOVIAM; ISSN: 0022-538X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The authors constructed **recombinant vaccinia** virus **vectors** for expression of the structural region of hepatitis C virus (**HCV**). Infection of mammalian cells with a **vector** (vv/HCV1-906) encoding C-E1-E2-NS2 generated major protein species of 22 kDa (C), 33 to 35 kDa (E1), and 70 to 72 kDa (E2), as observed previously with other mammalian expression systems. The bulk of the E1 and E2 expressed by vv/HCV1-906 was integrated into endoplasmic reticulum membranes as core-glycosylated species, suggesting that these E1 and E2 species represent intracellular forms of the **HCV** **envelope** proteins. **HCV** E1 and E2 formed E1-E2 complexes which were precipitated by either anti-E1 or anti-E2 serum and which sedimented at approx. 15 S on glycerol d. gradients. No evidence of intermol. disulfide bonding between E1 and E2 was detected. E1 and E2 were copurified to approx. 90% purity by mild detergent extraction, followed by chromatog. on Galanthus nivalus lectin-agarose and DEAE-Fractogel. Immunization of chimpanzees with purified E1-E2 generated high titers of anti-E1 and anti-E2 antibodies. Further studies demonstrated that purified E1-E2 complexes were recognized at high frequency by **HCV** + human sera and generated protective immunity in chimpanzees, suggesting that these purified **HCV** **envelope** proteins display native **HCV** epitopes.

L9 ANSWER 9 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1992:528131 CAPLUS

DOCUMENT NUMBER: 117:128131

TITLE: Hepatitis C virus asialoglycoproteins manufacture for vaccines or immunoassay

INVENTOR(S): Ralston, Robert O.; Marcus, Frank; Thudium, Kent B.; Gervase, Barbara A.; Hall, John A.

PATENT ASSIGNEE(S): Chiron Corp., USA

SOURCE: PCT Int. Appl., 28 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 8

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
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| WO 9208734 | A1 | 19920529 | WO 1991-US8272 | 19911107 |
| W: AU, CA, CS, FI, HU, JP, NO, PL, RO, SU | | | | |
| RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE | | | | |
| EP 414475 | A1 | 19910227 | EP 1990-309120 | 19900821 |
| EP 414475 | B1 | 19971210 | | |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE | | | | |
| AT 161041 | E | 19971215 | AT 1990-309120 | 19900821 |
| ES 2110411 | T3 | 19980216 | ES 1990-309120 | 19900821 |
| CA 2064705 | AA | 19910226 | CA 1990-2064705 | 19900822 |
| CA 2064705 | C | 19990406 | | |
| WO 9102820 | A1 | 19910307 | WO 1990-US4766 | 19900822 |
| W: AU, CA, JP | | | | |
| AU 9063449 | A1 | 19910403 | AU 1990-63449 | 19900822 |
| AU 655156 | B2 | 19941208 | | |
| JP 05502156 | T2 | 19930422 | JP 1990-512531 | 19900822 |
| JP 2001314192 | A2 | 20011113 | JP 2001-75114 | 19900822 |
| WO 9115771 | A1 | 19911017 | WO 1991-US2225 | 19910329 |
| W: AU, BB, BG, BR, CA, FI, GB, HU, JP, KP, KR, LK, MC, MG, MW, NO, PL, RO, SD, SU | | | | |
| RW: BF, BJ, CF, CG, CM, GA, ML, MR, SN, TD, TG | | | | |
| AU 9176510 | A1 | 19911030 | AU 1991-76510 | 19910329 |

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| AU 639560 | B2 | 19930729 | | |
| GB 2257784 | A1 | 19930120 | GB 1992-20480 | 19910329 |
| BR 9106309 | A | 19930420 | BR 1991-6309 | 19910329 |
| HU 62706 | A2 | 19930528 | HU 1992-3146 | 19910329 |
| HU 217025 | B | 19991129 | | |
| JP 05508219 | T2 | 19931118 | JP 1991-507636 | 19910329 |
| JP 2733138 | B2 | 19980330 | | |
| RO 109916 | B1 | 19950728 | RO 1975-92012 | 19910329 |
| PL 172133 | B1 | 19970829 | PL 1991-296329 | 19910329 |
| RU 2130969 | C1 | 19990527 | RU 1991-5053084 | 19910329 |
| EP 450931 | A1 | 19911009 | EP 1991-302910 | 19910403 |
| EP 450931 | B1 | 19960612 | | |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE | | | | |
| EP 693687 | A1 | 19960124 | EP 1995-114016 | 19910403 |
| EP 693687 | B1 | 19990728 | | |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE | | | | |
| AT 139343 | E | 19960615 | AT 1991-302910 | 19910403 |
| ES 2088465 | T3 | 19960816 | ES 1991-302910 | 19910403 |
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| ES 2134388 | T3 | 19991001 | ES 1995-114016 | 19910403 |
| CA 2095521 | AA | 19920509 | CA 1991-2095521 | 19911107 |
| AU 9190267 | A1 | 19920611 | AU 1991-90267 | 19911107 |
| AU 668078 | B2 | 19960426 | | |
| EP 556292 | A1 | 19930825 | EP 1992-900091 | 19911107 |
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| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE | | | | |
| JP 06504431 | T2 | 19940526 | JP 1992-500944 | 19911107 |
| HU 66063 | A2 | 19940928 | HU 1993-1336 | 19911107 |
| EP 842947 | A2 | 19980520 | EP 1997-120661 | 19911107 |
| EP 842947 | A3 | 20011212 | | |
| EP 842947 | B1 | 20040421 | | |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE | | | | |
| RU 2123528 | C1 | 19981220 | RU 1993-43621 | 19911107 |
| PL 175610 | B1 | 19990129 | PL 1991-300038 | 19911107 |
| AT 188220 | E | 20000115 | AT 1992-900091 | 19911107 |
| ES 2139591 | T3 | 20000216 | ES 1992-900091 | 19911107 |
| RO 115446 | B1 | 20000228 | RO 1993-626 | 19911107 |
| CA 2203443 | C | 20010828 | CA 1991-2203443 | 19911107 |
| JP 2001286290 | A2 | 20011016 | JP 2001-59335 | 19911107 |
| CZ 289006 | B6 | 20011017 | CZ 1993-824 | 19911107 |
| RU 2175657 | C2 | 20011110 | RU 1997-115378 | 19911107 |
| JP 2003093081 | A2 | 20030402 | JP 2002-199317 | 19911107 |
| JP 2003174875 | A2 | 20030624 | JP 2002-353148 | 19911107 |
| EP 1471073 | A2 | 20041027 | EP 2004-76119 | 19911107 |
| EP 1471073 | A3 | 20041201 | | |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE | | | | |
| FI 106317 | B1 | 20010115 | FI 1992-4349 | 19920928 |
| NO 9203839 | A | 19921119 | NO 1992-3839 | 19921001 |
| NO 310241 | B1 | 20010611 | | |
| FI 107803 | B1 | 20011015 | FI 1993-2025 | 19930505 |
| NO 9301680 | A | 19930628 | NO 1993-1680 | 19930507 |
| NO 304380 | B1 | 19981207 | | |
| LV 10344 | B | 19960220 | LV 1993-4381 | 19930531 |
| US 5679342 | A | 19971021 | US 1993-97853 | 19930727 |
| LT 3808 | B | 19960325 | LT 1993-1747 | 19931230 |
| US 5968775 | A | 19991019 | US 1995-438435 | 19950510 |
| US 5712087 | A | 19980127 | US 1995-440519 | 19950512 |
| US 6312889 | B1 | 20011106 | US 1995-440549 | 19950512 |
| FI 9701702 | A | 19970421 | FI 1997-1702 | 19970421 |
| FI 107804 | B1 | 20011015 | | |
| NO 9702213 | A | 19970514 | NO 1997-2213 | 19970514 |
| NO 304381 | B1 | 19981207 | | |
| PT 102022 | B | 20001229 | PT 1997-102022 | 19970626 |
| CZ 289923 | B6 | 20020417 | CZ 1997-2196 | 19970710 |
| JP 11071395 | A2 | 19990316 | JP 1998-103178 | 19980414 |
| JP 3207155 | B2 | 20010910 | | |
| GR 3031361 | T3 | 20000131 | GR 1999-402455 | 19990929 |
| GR 3032771 | T3 | 20000630 | GR 2000-400473 | 20000228 |
| JP 2004049235 | A2 | 20040219 | JP 2003-180211 | 20030624 |

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| US 1990-611419 | A | 19901108 |
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| US 1987-122714 | B2 | 19871118 |
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| US 1989-325338 | B2 | 19890317 |
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| US 1990-504352 | A | 19900404 |
| JP 1990-512531 | A3 | 19900822 |
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| US 1992-910760 | A3 | 19920707 |
| FI 1993-2025 | A | 19930505 |
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AB Two hepatitis C virus (**HCV**) **envelope** proteins (E1 and E2) are manufactured without sialylation. Expression of these genes in lower eukaryotes, or in mammalian cells in which terminal glycosylation is blocked, results in proteins similar to native **HCV** glycoproteins. When isolated by mannose-binding GNA (Galanthus nivalus agglutinin) lectin affinity, the E1 and E2 proteins aggregate into virus-like particles. Cells bearing a mannose receptor or asialoglycoprotein receptor are capable of being infected with **HCV** and of supporting culturing of the virus. E1 and E2 were produced in HeLa S3 cells inoculated with **recombinant Vaccinia** virus containing **HCV** gene fragments and purified using a GNA-agarose column.